Dellacha, J. M., Santomé, J. A., and Paladini, A. C. (1968), *Ann. N.Y. Acad. Sci. 148,* 313.
Geschwind, I. I., and Li, C. H. (1955), *in* The Hypophyseal Growth Hormone, Nature and Actions, Smith, R. W., Jr., Gaebler, D. H., and Long, C. N. H., Ed., New York, N.Y., McGraw-Hill, pp 25–58.
Gráf, L., Bajusz, S., Patthy, A., Barat, E., and Cseh, G. (1971), *Acta Biochim. Biophys. Acad. Sci. Hung. 6,* 415.
Gráf, L., Cseh, G., Nagy, I., and Kurcz, M. (1970), *Acta Biochim. Biophys. 5,* 299.
Gráf, L., and Li, C. H. (1974a), *Arch. Biochem. Biophys. 161,* 691.
Gráf, L., and Li, C. H. (1974b), *Biochem. Biophys. Res. Commun. 56,* 168.
Gray, W. R. (1967), *Methods Enzymol. 11,* 469.
Levine, L. S., Sonenberg, M., and New, M. I. (1973), *J. Clin. Endocrinol. Met. 37,* 607.
Li, C. H. (1954), *J. Biol. Chem. 211,* 555.
Li, C. H. (1956), *Advan. Protein Chem. 11,* 101.
Li, C. H. (1972), *Proc. Amer. Phil. Soc. 116,* 365.
Li, C. H., Dixon, J. S., Lo, T. B., Schmidt, K. D., and Pankov, Y. A. (1970), *Arch. Biochem. Biophys. 141,* 705.
Li, C. H., and Evans, H. M. (1944), *Science 99,* 183.
Li, C. H., Evans, H. M., and Simpson, M. E. (1945), *J. Biol. Chem. 159,* 353.
Li, C. H., Gordon, D., and Knorr, J. (1973), *Arch. Biochem. Biophys. 156,* 493.
Li, C. H., and Gráf, L. (1974), *Proc. Nat. Acad. Sci. U.S. 71,* 1197.
Offord, R. E. (1966), *Nature (London) 211,* 591.
Papkoff, H., and Li, C. H. (1958), *Biochim. Biophys. Acta 29,* 145.
Santomé, J. A., Dellacha, J. M., Paladini, A. C., Pena, C., Bicsoglio, M. J., Daurat, S. T., Poskeno, E., and Wolfenstein, C. E. M. (1973), *Eur. J. Biochem. 37,* 164.
Seavey, B. K., Singh, R. N. P., Lewis, U. J., and Geschwind, I. I. (1971), *Biochem. Biophys. Res. Commun. 43,* 189.
Sonenberg, M., Yamasaki, N., Kikutani, M., Swislocki, S., Levine, L., and New, M. (1972), *Excerpta Med., Int. Congr. Ser. 244,* 75.
Spackman, D. H., Stein, W. H., and Moore, S. (1958), *Anal. Chem. 30,* 1190.
Wallis, M. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 35,* 11.
Woods, K. R., and Wang, K. T. (1967), *Biochim. Biophys. Acta 133,* 369.
Yamasaki, N., Kangawa, K., Kobayashi, S., Kikutani, M., and Sonenberg, M. (1972), *J. Biol. Chem. 247,* 3874.
Yamasaki, N., Kiktani, M., and Sonenberg (1970), *Biochemistry 9,* 1107.
Yamashiro, D. (1964), *Nature (London) 201,* 76.

Cyclic Adenosine 3′,5′-Monophosphate Responses to Concanavalin A in Human Lymphocytes. Evidence that the Response Involves Specific Carbohydrate Receptors on the Cell Surface†

Leon R. Lyle‡ and Charles W. Parker*

ABSTRACT: Human peripheral blood lymphocytes, purified by isopycnic centrifugation, have been shown to undergo early increases in cAMP in response to concanavalin A (Con A). Changes in cAMP were demonstrable within 1 min suggesting that the response is initiated at the cell surface. This was confirmed by demonstrating that stimulation is also obtainable with Con A substituted polylysine–agarose beads, which cannot enter the cell. Control experiments with beads containing Con A indicated that the lectin remains attached to the beads under conditions in which stimulation of lymphocytes occurs. The cAMP response to Con A was blocked by methyl α-mannoside and methyl α-glucoside, which have affinity for the specific carbohydrate receptors on Con A, but not by other simple sugars. Taken together these observations indicate that Con A stimulates cAMP accumulation through its ability to interact selectively with carbohydrate receptors on the cell surface.

Stimulation of lymphocyte transformation by *Phaseolus vulgaris* phytohemagglutinin (PHA)[1] and other mitogenic lectins has been a widely studied model for induction of cell growth but the intriguing question of how the stimulation takes place has been difficult to elucidate. It was recently shown that PHA that is covalently bound to Sepharose can induce mitogenesis in mouse lymphocytes under conditions in which solubilization of mitogen is not demonstrable, indicating that a surface interaction is involved (Greaves and Bauminger, 1972). This creates a presumptive requirement for a secondary messenger inside the cell (Parker *et al.*, 1974).

Recent observations in human lymphocytes indicate that PHA produces changes in lymphocyte adenylate cyclase activity resulting either in a rise or a fall in cAMP (Smith *et*

† From the Washington University School of Medicine, Department of Medicine, St. Louis, Missouri 63110. *Received July 1, 1974.* Supported by a U. S. Public Health Service Training Grant AI00219.

‡ Present address: Mallinckrodt/Nuclear, Maryland Heights, Missouri 63145.

[1] Abbreviations used are: PHA, phytohemagglutinin; Con A, concanavalin A.

al., 1970, 1971; Parker, 1974; Parker *et al.*, 1974). The predominant early change is an increase in cAMP and immunofluorescence studies indicate the cAMP accumulates in or near the plasma membrane in a position where it might exert a modulating effect on plasma membrane function (Wedner *et al.*, 1972). The alterations in cAMP occur very rapidly, adding plausibility to the possibility that they may be involved in some of the early metabolic events in PHA stimulated cells. If the changes in cAMP are important in the initiation of lymphocyte replication, other lectins which stimulate mitogenesis might be expected to produce similar alterations in cyclic adenylate nucleotide concentrations. Studies with concanavalin A (Con A), a mitogenic protein which has a different carbohydrate specificity than PHA, would be of particular interest because agarose or polyacrylamide beads containing covalently bound Con A appear to have the same ability to stimulate lymphocyte transformation that PHA derivatized beads do (Betel and Van Den Berg, 1972; Ono *et al.*, 1973). In the present study it will be demonstrated that Con A produces very early alterations in lymphocyte cAMP metabolism and that the effect is dependent on its ability to interact with specific carbohydrate residues on the lymphocyte surface.

Experimental Section

Materials. Purified Con A was obtained from Miles Laboratories (Kankakee, Ill.) and was 98% homogeneous by gel electrophoresis. In some experiments Con A which had been further purified by affinity chromatography on Sephadex G-75 was used (generously provided by Dr. G. Kobayashi, Washington University School of Medicine). The later preparation was free of demonstrable contamination on the same analytical system. Con A was dissolved and diluted in 0.1 M NaCl. Concentrated solutions were stored at 4° or frozen for a maximum of 96 hr. *Phaseolus vulgaris* erythroagglutinating phytohemagglutinin (PHA) (MR-68) was obtained from the Burroughs Welcome, Co., Research Triangle Park, N.C. Reagent grade methyl α-D-glucoside, methyl α-D-mannoside, D-galactose, and *N*-acetyl-D-glucosamine were obtained from the Sigma Chemical Company (St. Louis). Con A substituted Sepharose beads, prepared by the reaction of purified Con A with cyanogen bromide activated Sepharose, were obtained from Miles-Yeda Ltd. (Rehovot, Israel). Control (unsubstituted) Sepharose beads were obtained from the Sigma Chemical Company. Con A–Sepharose was also prepared by reacting washed Sepharose 4B (Sigma Chemical Company) activated with cyanogen bromide with polylysine, as described by Wilchek, 1973, followed by conjugation to Con A in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Ott Chemical Co., Muskegon, Mich.); 50 mg of Con A, trace labeled with ^{125}I, or unlabeled, was reacted with 5 ml of polylysine-substituted beads and 50 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in 5 ml of water at pH 5.5. The suspension was gently stirred at 22° for 16 hr. During the reaction the pH was monitored intermittently and readjusted to 5.5 as required. The preparation was then thoroughly washed and counted or the supernatant and washes analyzed for protein. By radioactivity the Con A–polylysine–Sepharose (Con A–poly(Lys)–S) contained 3.5 (±0.2 SEM) mg of Con A/ml of beads (two preparations). As a control ^{125}I or unlabeled bovine serum albumin was similarly conjugated to polylysine–Sepharose; the product contained an estimated 4.2 mg of bovine serum albumin/ml of beads. Before each experiment trace amounts of solubilized lectin were removed by packing the beads in a column and washing them continuously with Gey's solution for 2–3 hr in the cold.

Cell Purification. Purified human lymphocytes were obtained by dextran sedimentation of heparinized human peripheral blood followed by isopycnic centrifugation in a Ficoll-Hypaque gradient as described elsewhere (Eisen *et al.*, 1972; Parker and Smith, 1973). These preparations contain on average 95 (range 91–98) lymphocytes, 3 neutrophiles, and 2 monocytes per 100 nucleated cells as determined on stained fixed smears (300–500 cells counted). There are also a variable but small number of erythrocytes and platelets as determined by phase contrast microscopy.

cAMP Studies. Purified lymphocytes were suspended in Gey's solution (Gey and Gey, 1936). Gey's solution contains 8 g of NaCl, 0.375 g of KCl, 0.226 g of $Na_2HPO_4 \cdot 7H_2O$, 0.025 g of KH_2PO_4, 2 g of dextrose, 0.25 g of $NaHCO_3$, 0.21 g of $MgCl_2 \cdot 6H_2O$, and 0.275 g of $CaCl_2$ in final volume of 1 l. (final pH 7.6). In the usual experiment 0.01 ml of Con A solution or buffer was added to 0.05 ml of lymphocyte suspension at a density of 2×10^7 cells/ml (1×10^6 lymphocytes/tube). In some experiments the same number of lymphocytes were incubated in a large volume of medium and the volume of Con A solution or buffer was increased proportionately. The cell suspension was mixed at room temperature and incubated at 37° for 1–15 min in a water-saturated atmosphere without shaking. At the end of the incubation, the cells were centrifuged at 2500 rpm for 2 min at room temperature as described previously (Smith *et al.*, 1971). After the medium had been decanted the cell pellet was frozen in ethanol–Dry Ice and stored at −70° until assay. In a few experiments the lymphocyte suspension was centrifuged immediately before incubation, Con A was added in a small volume of Gey's solution directly, and the reaction was terminated by freezing the cell buffer mixture in liquid nitrogen (without further centrifugation). cAMP was measured by radioimmunoassay (Steiner *et al.*, 1969) following extraction of the frozen lymphocyte pellets by boiling for a few minutes in 0.05 M acetate buffer (pH 6.2), sonication, and centrifugation to remove the insoluble residue. The extraction procedure was verified by cAMP recovery studies in which 0.05–20 pmol of cAMP was added to the lymphocyte pellet before freezing and extraction and shown to give the expected increment in measured cAMP. There was good correspondence between cAMP values in Cl_3CCOOH extracted and boiled samples both in unstimulated lymphocytes and in lymphocytes stimulated with PGE_1, catecholamine, and PHA. Validation of the sensitivity and specificity of the radioimmunoassay and the demonstration that it is applicable to human lymphocytes have been given previously (Steiner *et al.*, 1969, Smith *et al.*, 1971). The quantities of Con A and carbohydrates used in the investigation were shown to not alter the cAMP immunoassay. Methyl α-glucoside or methyl α-D-mannoside, where used, was mixed with the cells prior to the addition of Con A.

Results

Effects of Con A on Lymphocyte cAMP Concentrations. The results of short incubation (1–15 min) experiments in Gey's solution indicated that the predominant effect of Con A was to produce a rise in intralymphocytic cAMP concentrations (a >60% increase in cAMP in 19 of 20 experiments at 1.6×10^7 cells/ml), particularly at concentrations in the 6–30 μg/ml range. The time course of the cAMP response

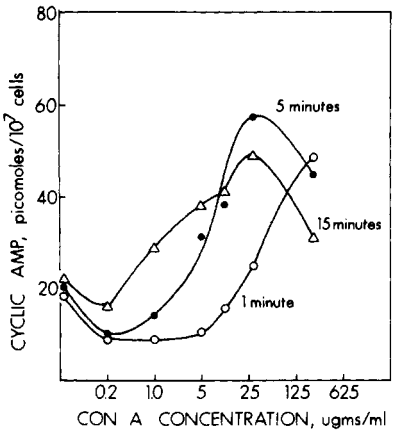

FIGURE 1: Time course of the lymphocyte cAMP response to Con A. 1 × 10^6 lymphocytes was centrifuged and incubated as a pellet at 37° in the presence of 0.04 ml of buffer (Gey's solution) or Con A in buffer for various time periods at 37°. The reaction was stopped by snap freezing the incubated mixtures in liquid nitrogen. The data are from a single experiment with incubation times at 1 (○), 5 (●), and 15 (△) min.

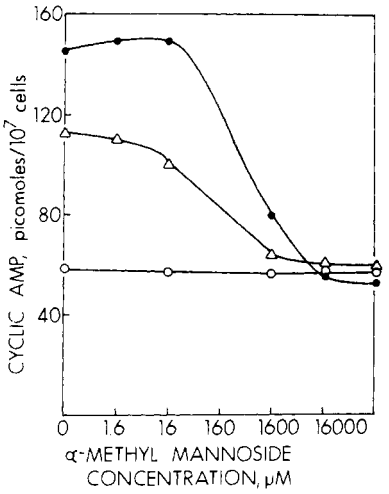

FIGURE 2: Methyl α-D-mannoside inhibition of the lymphocyte cAMP response to Con A. 1 × 10^6 purified lymphocytes in a total volume of 0.06 ml of Gey's solution was incubated in the presence and absence of methyl α-D-mannoside and Con A for 10 min at 37°. In tubes which contained methyl α-D-mannoside the sugar was added to the lymphocyte suspension prior to addition of Con A. The incubation conditions were 30 μg/ml of Con A (●), 6 μg/ml of Con A (△), and no Con A (○). The concentrations of methyl α-D-mannoside used produced no more than 20% inhibition of the lymphocyte cAMP response to PHA (at concentrations of PHA producing a maximum increase in cAMP), see Figure 3.

was studied (five experiments) in cell pellets exposed to small volumes of buffer or Con A solution and rapidly frozen in liquid nitrogen (Figure 1). A response was demonstrable within 1 min with a fall in cAMP in the low and mid dose range (0.2–15 μg/ml) and a rise in the mid to high dose range (30–200 ml). After 5 min the curve had shifted with positive responses at 5 and 16 μg/ml of Con A as well as at higher Con A concentrations and a new maximum at 30 μg/ml of Con A; at 0.2 μg/ml a modest fall in cAMP was usually seen, even after 10 or 15 min. The same results were obtained with a commercial preparation of Con A which was about 98% pure by gel electrophoresis and with Con A which had been further purified by affinity chromatography and was free of demonstrable contamination; thus the change in lymphocyte cAMP levels was not due to trace

TABLE I: Specificity of Carbohydrate Inhibition of the Lymphocyte cAMP Response to Con A.[a]

Con A Concn (μg/ml)	Carbohydrate Inhibitor[b]	cAMP pmol/10^7	% Inhibition[c]
		60	
30		118	
30	Methyl α-D-mannoside	51	100
30	Methyl α-D-glucoside	48	100
30	*N*-Acetyl-D-glucosamine	96	38
30	D-Galactose	92	45
	Methyl α-D-mannoside	58	
	Methyl α-D-glucoside	56	
	N-Acetyl-D-glucosamine	60	
	D-Galactose	56	

[a] 1 × 10^6 purified lymphocytes in a total volume of 0.06 ml of Gey's solution was incubated in the presence and absence of 30 μg/ml of Con A and 50 mM carbohydrate for 10 min at 37°. [b] In tubes containing carbohydrate the solution of carbohydrate was added to the lymphocyte suspension prior to the addition of Con A. [c] Per cent inhibition of the increase obtained with Con A alone.

contaminants in the Con A. Similar cAMP responses were obtained when the cell density was varied from 4 × 10^6 to 3.2 × 10^7 lymphocytes/ml (not shown). The response varied with the Con A concentration rather than the total quantity of Con A present per tube.

Since methyl α-D-glucoside and methyl α-D-mannoside competitively inhibit the binding of Con A to lymphocytes (Sharon and Lis, 1972; Chase and Miller, 1973) they can be used to evaluate whether the change in cAMP in the presence of the lectin involves specific carbohydrate receptors. Figure 2 examines the relationship between methyl α-D-mannoside concentration and inhibition of the lymphocyte cAMP response to Con A; 1.6 mM concentrations of the monosaccharide largely (>70%) inhibited the response and 32 mM concentrations were completely inhibitory. Similar results were obtained with a methyl α-glucoside (45 and 92% inhibition at 1.6 and 32 mM concentrations, respectively (not shown)). The somewhat greater effectiveness of methyl α-D-mannoside as an inhibitor is what would be predicted from equilibrium dialysis and precipitin inhibition studies which indicate that methyl α-D-mannoside has a greater affinity for the protein than the corresponding glucoside (Goldstein *et al.*, 1973). The ability of other sugars to inhibit the cAMP response is shown in Table I. Sugars which interact poorly with Con A (D-galactose and *N*-acetyl-D-glucosamine) produced <50% inhibition of the response, even at 50 mM concentrations.

The specificity of the methyl α-D-mannoside inhibition was further evaluated by carrying out parallel studies with PHA which also raises cAMP in human lymphocytes (Smith *et al.*, 1971) but interacts with different carbohydrate receptors on the cell surface. As shown in Figure 3, methyl α-D-mannoside does not inhibit the cAMP response to PHA.

If the effect of Con A on lymphocyte cAMP concentrations is due to binding of the mitogen at the lymphocyte surface it might be possible to demonstrate that the change in cyclic nucleotide concentration can be reversed by sugars

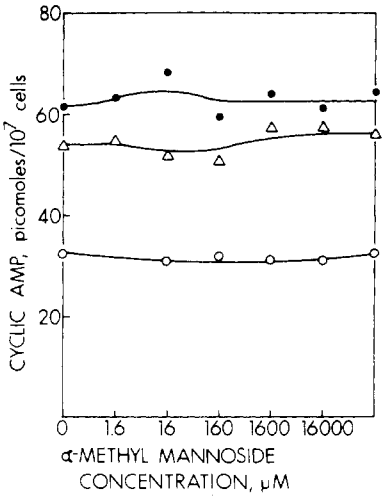

FIGURE 3: Evaluation of methyl α-D-mannoside as an inhibitor of the lymphocyte cAMP response to PHA. 1 × 10^6 purified lymphocytes in a total volume of 0.06 ml of Gey's solution was incubated in the presence and absence of methyl α-D-mannoside and PHA for 10 min at 37°. In tubes which contained methyl α-D-mannoside the sugar was added to the lymphocyte suspension prior to the addition of Con A. The incubation conditions were 30 μg/ml of PHA (●), 6 μg/ml of PHA (△), and no PHA (○).

that interact specifically with Con A. Indeed, as shown in Table II, the changes in cAMP did not persist in cells preincubated with Con A and then exposed to methyl α-D-glucoside for an additional 10 min at 37°. Cuatrecasas (1973) has previously demonstrated an ability of simple sugars to dissociate Con A from fat cells and speculated as to why the dissociation occurs as quickly as it does.

The reversibility of the increase in cAMP when Con A containing cells were exposed to methyl α-D-glucose (see above, Table II) strongly suggested that modulation of cAMP accumulation by the lectin was taking place at the cell surface. This possibility was further examined using Con A insolubilized on Sepharose beads. With biologically active proteins that are covalently attached to Sepharose, any metabolic effects that are produced should be at the cell surface since Sepharose particles are too large to be internalized (Cuatrecasas, 1969). Conjugates of Con A attached directly to Sepharose appeared to produce a modest increase in cell cAMP concentrations in several experiments but leaked significant amounts of Con A, making interpretation difficult. Wilchek (1973) and Sica *et al.* (1973) have suggested that the stability of protein–Sepharose conjugates can be improved by placing a poly(amino acid) or protein bridge between the protein and the agarose. The formation of a secondary attachment through a macromolecular spacer also increases the physical separation of the protein from the gel which might increase its biological reactivity. We therefore attached Con A to polylysine–Sepharose. The Con A–polylysine–Sepharose beads (Con A–poly(Lys)–S beads) were stable under the incubation conditions used (see below) and at 6 and 2% bead concentration increases in lymphocyte cAMP occurred with blocking of the response by methyl α-D-mannoside (Table III). The increase in cAMP was less marked than with soluble Con A, presumably because of steric limitations on the ability of Con A to interact with cells when it is attached to beads.

A number of lines of evidence made it highly unlikely that elution of Con A could explain that the increase in lymphocyte cAMP concentrations obtained with Con A–poly(Lys)–S beads. (1) Under the conditions used the quantities of soluble Con A required for stimulation of cAMP accumulation were relatively high (1.2 μg/ml, Table III). Supernatants from mixtures of Con A–poly(Lys)–S beads and lymphocytes failed to produce increases in cAMP in fresh lymphocytes (Table III) (in contrast to supernatants from soluble Con A–lymphocyte mixtures). In experiments with ^{125}I labeled Con A–poly(Lys)–S essentially no radioactivity (<0.2%) was present in the supernatant after incubation at 37° for 15 min in the presence of lymphocytes. (3) Short incubation times were used and the beads had been rigorously washed immediately prior to the experiment. (4) Microscopic analysis of lymphocytes incubated in 2 or 6% Con A–poly(Lys)–S suspensions revealed cells adhering directly to beads. In incubation mixtures containing soluble Con A and 2 or 6% unsubstituted Sepharose, the cells were adhering to one another and not to the beads.

TABLE II: Reversibility of the cAMP Response to Con A with Methyl α-D-Glucoside.[a]

Con A Concn (μg/ml)	Final Methyl α-Glucoside Concn (mM)	cAMP pmol/10^7 cells	% Inhibition[b]
Control		20	
120		84	
120	45	26	93
20		35	
20	45	23	80
4		32	
4	45	21	91
Control	45	21	

[a] 1 × 10^6 lymphocytes in 0.06 ml of Gey's solution was preincubated for 5 min at 37° in the presence and absence of Con A; they were then diluted either with 8 volumes of Gey's solution, or with 8 volumes of 50 mM methyl α-glucoside in Gey's (prepared by mixing 6.4 volumes of ordinary Gey's solution and 1.6 volumes of 250 mM methyl α-glucoside) and incubated for another 10 min at 37°. Similar cAMP values were obtained when a mixture of 6.4 volumes of Gey's solution and 4.6 volumes of 250 mM NaCl was used instead of Gey's solution for dilution of Con A or buffer containing tubes (not shown). [b] Per cent inhibition of the increase obtained with Con A alone.

Discussion

This study demonstrates an ability of Con A to modulate cAMP accumulation in purified human lymphocytes. The tempo and magnitude of the changes are similar to those described previously with phytohemagglutinin (PHA) (Smith *et al.*, 1971; Parker *et al.*, 1974), indicating a possible common mechanism of action. The effect on cAMP accumulation involves specific carbohydrate receptors as demonstrated by the ability of simple sugars that inhibit Con A binding to lymphocytes (methyl α-D-glucoside and methyl α-D-mannoside) to also inhibit the cAMP response. The specificity of the inhibition is indicated by the failure of the two sugars to inhibit the cAMP response to PHA, which has a different carbohydrate specificity than Con A and the absence of comparable inhibition with galactose and *N*-acetylglucosamine.

TABLE III: Stimulation of Lymphocyte cAMP Accumulation with Con A–Sepharose–Polylysine Conjugates.[a]

Stimulator	Final Concn	Methyl α-D-Mannoside Concn (mM)	cAMP pmol/107 cells	cAMP Stimulation by Supernatant[b]
Buffer			19 (±1)	–
Con A–poly(Lys)–Sepharose	6%		36 (±3)[c]	–
	2%		35 (±1)[c]	–
	0.6%		14 (±5)	–
	0.2%		22 (±3)	–
Con A–poly(Lys)–Sepharose	6%	50	23 (±3)	
	2%	50	21 (±2)	
	0.6%	50	20 (±4)	
	0.2%	50	22 (±0)	
BSA–poly(Lys)–Sepharose[d]	6%		15 (±3)	
	2%		16 (±4)	
	0.6%		20 (±2)	
	0.2%		20 (±1)	
Soluble Con A	30 μg/ml		84 (±15)[c]	+
	6 μg/ml		60 (±7)[c]	+
	1.2 μg/ml		22 (±5)	–
	0.2 μg/ml	1	22 (±3)	–

[a] 3×10^7 lymphocytes in a volume of 0.15 ml of Gey's solution was incubated in the presence and absence of Con A–poly(Lys)–Sepharose, BSA–poly(Lys)–Sepharose, soluble Con A, and methyl α-D-mannoside for 10 min at 37°. Given as mean (±SEM) for two experiments. [b] At the conclusion of the experiment the supernatant solutions were aspirated from the centrifuged lymphocyte pellets and analyzed with fresh lymphocytes for their ability to stimulate cAMP accumulation (1×10^6 lymphocytes in 0.01 ml in Gey's solution and 0.05 ml of supernatant, incubated for 10 min at 37°). Supernatant solutions from cells incubated at 30 μg/ml concentrations of soluble Con A produced 42 and 28% increases in lymphocyte cAMP concentrations, respectively. None of the other supernatant solutions produced a >10% increase in cAMP. [c] $P < 0.01$. [d] BSA, bovine serum albumin.

The biochemical basis for the change in lymphocyte cAMP concentration in response to Con A is currently under study. By analogy with PHA an action at the level of adenylate cyclase would be predicted. PHA has been shown to stimulate adenylate cyclase in crude homogenates and plasma membrane rich fractions from human lymphocytes (Smith *et al.*, 1971; Snider and Parker, unpublished observations) and to not change the activity of cAMP phosphodiesterase in crude enzyme preparations from the same cells (Lagarde and Colobert, 1972). Observations in progress with Con A in homogenates of human and rat lymphocytes indicate that Con A also has a stimulatory effect on lymphocyte adenylate cyclase (Snider, Wedner and Parker, unpublished observations). The tentative conclusion that Con A is acting through adenylate cyclase is reinforced by recent studies in isolated rat fat cells where Con A has been shown to alter glucose uptake (Czech and Lynn, 1973; Cuatrecasas and Tell, 1973) and to stimulate or inhibit adenylate cyclase in these cells depending on the Con A concentration used (Cuatrecasas and Tell, 1973).

It can be inferred from the rapidity of the change in cAMP at high concentrations of Con A (within 1 min) and the ready availibility of Con A receptors on the lymphocyte exterior that the initial stimulus to cAMP accumulation is most likely at the lymphocyte surface. Three additional lines of evidence indicate an action at this level. (1) Con A–Sepharose conjugates produce rises in cAMP under conditions in which elution of Con A from the beads cannot be demonstrated. (2) The rise in cAMP in response to Con A can be reversed when methyl α-glucoside is added to the medium after stimulation is already underway. (3) As discussed elsewhere immunofluorescence studies with anti-cAMP antibody indicate that Con A promotes a marked accumulation of cAMP in localized patches at or near the lymphocyte plasma membrane (Parker *et al.*, 1974). The location, rapidity, and specificity of the cAMP response to Con A suggest that cAMP may be involved in some of the early changes in plasma membrane function (K^+, Ca^{2+}, glucose, and nucleoside transport) that occur during lymphocyte stimulation by this agent.

Despite the early changes in cAMP in PHA and Con A stimulated lymphocytes the role of cAMP in the ultimate mitogenic response remains to be elucidated. Some of the problems in interpretation are the following. (1) At concentrations of Con A above 1 μg/ml an increase in cAMP is obtained; at concentrations below 1 μg/ml a fall in cAMP is frequently seen. The fall in cAMP may relate to the observations of Cuatrecasas and Tell (1973) referred to above that in fat cell membranes intermediate and low concentrations of Con A inhibit adenylate cyclase activity. (2) When cells are incubated in medium with lectins for many hours at concentrations which produce early rises in cAMP a later fall in the cyclic nucleotide is frequently seen, suggesting that an initial positive response has been converted to a negative response (Parker *et al.*, 1974). (3) Con A is similar to PHA in that it produces a maximal cAMP response at concentrations of lectin above the mitogenic optimum. However this may be due at least in part to lymphocyte heterogeneity. The human lymphocytes utilized in this study contain about 65–80% T lymphocytes and 20–35% B lymphocytes. Most of the mitogenic response to PHA and Con A is in T cells. When purified T cells are studied (both in simple

buffer and tissue culture media) the maximal cAMP response to PHA or Con A is now in the optimal mitogenic range and there is no longer a cAMP response at high doses of lectin (Parker *et al.*, 1974). Thus, in the lymphocyte subpopulation which is most active mitogenically there is no longer a discrepancy between optimal mitogenic concentration and the early cAMP response.

In addition to cAMP large (10–50-fold) increases in cGMP have been reported in response to Con A and PHA in human lymphocytes (Hadden *et al.*, 1972). Schumm *et al.* (1974) have observed similar large increases in cAMP in unpurified rat blood cells exposed to PHA. In our own experiments which will be reported in detail elsewhere increases in cGMP are much less impressive (less than two-fold) and often completely absent. While we have no explanation for this discrepancy it is clear that both cyclic nucleotides deserve careful study as possible mediators of lectin action. Whether either cyclic nucleotide, acting alone, can reproduce the entire biochemical spectrum of mitogenesis remains to be established. Both cAMP and cGMP have been reported to produce small increases in DNA synthesis in human and animal lymphocytes but the response is far below that obtained with PHA or Con A (Parker *et al.*, 1974).

Acknowledgment

We thank Miss Joan Poll, Mrs. Mary Huber, and Miss Mary Baumann for their expert technical assistance.

References

Betel, I., and Van Den Berg, K. J. (1972), *Eur. J. Biochem. 30,* 571.

Chase, P. S., and Miller, F. (1973), *Cell. Immunol. 6,* 132.

Cuatrecasas, P. (1969), *Proc. Nat. Acad. Sci. U. S. 63,* 450.

Cuatrecasas, P. (1973), *Biochemistry 12,* 1312.

Cuatrecasas, P., and Tell, G. P. E. (1973), *Proc. Nat. Acad. Sci. U. S. 70,* 485.

Czech, M. P., and Lynn, W. S. (1973), *Biochim. Biophys. Acta 297,* 368.

Eisen, S. A., Wedner, H. J., and Parker, C. W. (1972), *Immunol. Commun. 1,* 571.

Gey, G. O., and Gey, M. K. (1936), *Amer. J. Cancer 27,* 45.

Goldstein, I. J., Reichert, C. M., Misaki, A., and Gorin, P. A. J. (1973), *Biochim. Biophys. Acta 317,* 500.

Greaves, M. F., and Bauminger, S. (1972), *Nature (London), New Biol. 235,* 67.

Hadden, J. W., Hadden, E. M., Haddox, M. K., and Goldberg, N. D. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 3024.

Lagarde, A., and Colobert, L. (1972), *Biochim. Biophys. Acta 276,* 444.

Lindahl-Kiessling, K. (1972), *Exp. Cell Res. 70,* 17.

Ono, M., Maruta, H. and Mizuno, D. (1973), *J. Biochem. 73,* 235.

Parker, C. W. (1974) *in* Cyclic AMP, Cell Growth and the Immune Response, Braun, W., Lichtenstein, L. and Parker, C. Ed., New York, N.Y., Springer-Verlag New York, pp 35–44.

Parker, C. W., and Smith, J. W. (1973), *J. Clin. Invest. 52,* 48.

Parker, C. W., Sullivan, T. J., and Wedner, H. J. (1974), *in* Advances in Cyclic Nucleotide Research, New York, N.Y., Raven Press (in press).

Schumm, D. E., Morris, H. P., and Webb, T. E. (1974), *Eur. J. Cancer 10,* 107.

Sharon, N., and Lis, H. (1972), *Science 177,* 949.

Sica, V., Parikh, I., Nola, E., Puca, G., and Cuatrecasas, P. (1973), *J. Biol. Chem. 248,* 6453.

Smith, J. W., Steiner, A. L., and Parker, C. W. (1970), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29,* 369.

Smith, J. W., Steiner, A. L., and Parker, C. W. (1971), *J. Clin. Invest. 50,* 442.

Steiner, A. L., Kipnis, D. M., Utiger, R., and Parker, C. W. (1969), *Proc. Nat. Acad. Sci. U. S. 64,* 367.

Wedner, H. J., Bloom, F. E., and Parker, C. W. (1972), *Clin. Res. 20,* 798.

Wilchek, M. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 33,* 70.